Risk factors for Kaposi's sarcoma-associated herpesvirus infection among HIV-1-infected pregnant women in the USA

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Objectives: We sought to identify risk factors for infection with the Kaposi's Sarcoma-associated herpesvirus (KSHV) among pregnant women and to examine a reported association of KSHV with injecting drug use (IDU) and hepatitis C virus (HCV) infection.

Design: Cross-sectional evaluation of questionnaire data and KSHV and HCV seroprevalence in the Women and Infants Transmission Study.

Methods: In sera collected from HIV-1-infected pregnant women (n = 887) and, at age 12 months, their offspring (n = 900) at six sites in the USA and Puerto Rico, KSHV and HCV antibodies were detected with sensitive and specific enzyme immunoassays. Risk of KSHV was estimated by the unadjusted and adjusted odds ratio (OR_{adj}) and 95% confidence interval (CI). The geographic referent sites were Chicago and Boston.

Results: Forty-seven (5.3%) of the women and three (0.3%) of the infants were KSHV seropositive. In univariate and multivariate analyses, KSHV in the women was associated with enrollment in Puerto Rico, Houston or Brooklyn ($OR_{adj,}$ 4.3; 95% CI, 1.8–10.4) or Manhattan ($OR_{adj,}$ 9.8; 95% CI, 3.7–25.6); non-completion of high school ($OR_{adj,}$ 1.8; 95% CI, 0.9–3.4); the number of sexually transmitted diseases ($OR_{adj,}$ 1.4; 95% CI, 1.0–1.9 per disease); and especially with IDU and HCV infection ($OR_{adj,}$ 3.5; 95% CI, 1.5–7.9).

Conclusions: Transmission of KSHV by blood inoculation may be highly inefficient, but our data support the hypothesis that it does occur. Large formal studies to evaluate whether KSHV transmission occurs via transfusion are needed to inform decisions regarding screening volunteer blood donors to protect the blood supply.

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Introduction

Kaposi's sarcoma (KS) is caused by a herpes virus that was discovered in 1994 and termed human herpesvirus 8 or KS-associated herpes virus (KSHV) [1]. Although KSHV shares substantial homology and some biological properties with Epstein–Barr virus (EBV), it has a markedly lower prevalence in most populations, with a seroprevalence of approximately 3.3% among blood donors in the USA [2]. In sub-Sarahan Africa, KSHV seroprevalence rates of 20% among blood donors and 30–50% among various populations have been reported [3–5].

KSHV seroprevalence also is high among homosexual men in the USA, ranging from 9 to 13% without HIV infection to 35–49% with HIV infection [4–7]. These KSHV seroprevalence rates, which mirror a high incidence of AIDS-associated KS [8] are particularly high in men who have had numerous homosexual partners or a sexually transmitted disease (STD) [6,7].

Nonetheless, the modes of transmission of KSHV remain poorly defined. KS occurs in prepubertal children indicating that non-sexual transmission must occur [9] and KSHV age-specific seroprevalence rates suggest that transmission occurs among children [10,11]. Transmission may occur through kissing or other close personal contact with saliva, as KSHV, like EBV, can be detected at relatively high levels in saliva [12,13].

Among non-African women in the USA and England, KSHV seroprevalence rates of 3.4–9% were found among women with or at high risk of an STD [5,14,15]. Among such women, Cannon *et al*. recently estimated the risk for KSHV was increased approximately 2-fold with positive syphilis serology [16] compatible with a previous estimate from England [15]. More remarkably, those investigators found that KSHV infection was increased significantly 1.7-fold with hepatitis C virus (HCV) seropositivity or approximately 3-fold with daily injecting drug use (IDU) [16]. The associations of KSHV with HCV and IDU were particularly apparent among women with few sexual risk factors.

If KSHV is transmitted by IDU, it could be transmitted by blood transfusion, implying that procedures to ensure the safety of the blood supply may be required. Thus, we sought to corroborate or refute the association of KSHV with IDU and HCV in another population of women.

Methods

Study population

The Women and Infants Transmission Study (WITS) has been described [17]. Briefly, HIV-1-infected women in four states and Puerto Rico were evaluated up to three times during pregnancy and at delivery. Informed consent was obtained from each woman in accordance with the institutional review board requirements at each site. At each visit, detailed medical and behavioral questionnaires were administered, a physical examination was performed, and blood samples were collected. The infants were examined and blood samples were obtained within 7 days of birth; at ages 1, 2, 4, 6, 9, 12 and 18 months; and every 6 months thereafter. The current analysis included 887 mothers enrolled between December 1989 and November 1997, each of whom had a frozen serum sample that had been drawn near parturition, as well as a frozen serum sample from their infants (n = 900, including 13 twin pairs) that had been drawn at approximately age 12 months (median, 12.5 months; range, 11.0–26.3 months).

At enrollment and at each follow-up, the participant's medical record was abstracted. Trained nurses used a structured interview questionnaire to obtain demographic information, past medical history including STD, lifetime male sex partners, age at first sexual intercourse, use of alcohol, cigarettes, marijuana, antiretroviral therapy, cocaine, heroin, methadone, and other drugs. Urine for toxicology analysis was collected from women at enrollment, during labor or immediately postpartum, and 6 months postpartum.

Recreational drug use

Urine specimens, shipped on dry ice overnight to a single reference laboratory (The Center for Human Toxicology, Utah, USA) certified by the National Laboratory Certification Program of the National Institute on Drug Abuse, were analyzed for drugs or metabolites of cocaine, heroin/opiates, methadone, marijuana, alcohol, and other drugs. The enzyme-multiplied immunoassay technique (EMIT) was used to screen for methadone, while radioimmunoassay (RIA) methodology (Roche Diagnostics, Branchberg, New Jersey, USA) was used for detection of other drugs. Positive screening assays were followed by confirmatory gas chromatography and mass spectrometry unless use of the specific drug was reported by the woman or confirmed at an earlier visit by gas-chromatography/mass spectrometry.

IDU was based on reported lifetime injecting drug history at enrollment or pregnancy visits regardless of drug types. Non-injecting hard drug use refers to positive self-report, urine toxicology, or both, for one or more of the following: cocaine, heroin/opiates, or methadone without reported injection. Non-use was defined as negative by self-report and urine toxicology or by negative self-report alone if urine toxicology results were missing.

Laboratory studies

KSHV antibodies were detected by second-generation enzyme immunoassay (EIA) for reactivity against the K8.1 antigen [18,19]. K8.1 has no homology with any region of EBV or other human herpesvirus genomes. Used alone, our first-generation K8.1 EIA had an estimated sensitivity of 78% and specificity of 98%, as determined by comparison of postulated gold-standard positive (KS) and negative (hemophilic) sera [18]. Our second-generation EIA, which was used for this study, had consistently high sensitivity [point estimate, 100%; 95% confidence interval (CI), 96–100%] and specificity (point estimate, 98%; 95% CI, 94–100%) across populations in a latent class analysis [19]. KSHV-seropositive sera were defined as those with K8.1 EIA optical density greater than 1.0; those with lower optical densities were seronegative.

Each woman's HCV status was defined by detection of HCV antibodies with a second- or third-generation EIA (Ortho Diagnostic Systems, Raritan, New Jersey, USA). The positive predictive value of these assays in this population was 98.7% [89.2% with HCV RNA detected by quantitative reverse trascriptase–PCR (Amplicor Monitor HCV Assay, Roche Diagnostics) plus 9.5% with HCV antibodies detected by second-generation recombinant immunoblot assay (RIBA, Ortho Diagnostic Systems)] [20].

Medical history, examination, record abstraction and laboratory data were used to diagnose STD during pregnancy. At enrollment and again at week 34 ($\pm$ 4 weeks) antepartum, sera were tested for syphilis with the rapid plasma reagin (RPR) or venereal disease research laboratory (VDRL) assay, with reactive samples confirmed by fluorescent treponemal antibody (FTA) or *Treponema pallidum* hemagglutination assay (TPHA). At the same visits, appropriate cervical and vaginal specimens were obtained for *Neisseria gonorrhoeae* culture on modified Thayer Martin media, *Chlamydia trachomatis* assessment using the Microtrak slide test, and *Trichomonas vaginalis* culture in Diamond's media. In addition, clinically obtained laboratory results were abstracted. A specific STD was defined as present if indicated by any of the history, examination, or laboratory assessments. Absence of that STD was defined as no indication of its presence.

Statistical analysis

KSHV seroprevalence was calculated for the 900 infants (874 singletons and 13 twin pairs). Among women, logistic regression was used to compute univariate and stratified odds ratios (OR) and 95% CI for demographic, sexual, IDU-associated, and serological variables associated with KSHV seropositivity. Chi-square and Fisher's exact test were used to test for statistically significant associations. Multivariate logistic regression was used to determine the independence and adjusted OR (OR_{adj}) of variables associated with KSHV. Variables with $P < 0.10$ were retained in the multivariate model. An EM algorithm [21] was used to estimate the OR with correction for imperfect sensitivity and specificity of the KSHV K8.1 EIA. Confounding by variables was evaluated by stratification and magnitude of the change in estimate. All statistical tests were two-sided.

Results

KSHV seroprevalence and agreement in mother–infant pairs

KSHV antibodies were detected in 47 (5.3%) of 887 women whose sera were drawn near the time of delivery. Three (0.3%) of their 900 infants had KSHV antibodies detected at approximately age 12 months. Only one of the three seropositive infants had a seropositive mother.

Risk factors for KSHV in women

KSHV seroprevalence differed significantly among the six enrollment sites. It was low (2.4–2.8%) in Chicago and Boston; intermediate (5.4–6.0%) in Puerto Rico, Houston, and Brooklyn; and high (15.1%) in Manhattan (Table 1). KSHV seroprevalence was 6-fold higher (OR, 6.1; 95% CI, 2.1–17.8) in Manhattan compared to Chicago. Seroprevalence did not differ by ethnicity (4.6–5.7%) or age ($P_{trend} = 0.18$), but it was increased approximately 2-fold with lower education or income.

Consistent with eligibility, all of the women were infected with HIV-1. KSHV seroprevalence was unrelated to the women's stage of and treatment for HIV-1 and AIDS. Specifically, seroprevalence ranged from 3.9% to 6.4% across categories of HIV-1 disease, CD4 lymphocytes, HIV-1 viral load, antiretroviral drug therapy, and transmission of HIV-1 to the infant (Table 1).

KSHV seroprevalence was not related to the reported lifetime number of male sexual partners nor with oral or anal sex during the index pregnancy (Table 1). KSHV seroprevalence was increased among women who were diagnosed during the index pregnancy with chancroid (OR, 5.3; 95% CI, 1.1–26.2), syphilis (OR, 2.3; 95% CI, 1.1–5.0), or genital warts (OR, 2.0; 95% CI, 1.0–3.9). KSHV seroprevalence increased directly with the number of STD: 3.8% with none, 4.7% with one, 7.3% with two, and 10.3% with at least three ($P_{trend} = 0.01$).

KSHV seroprevalence was increased 2-fold (95% CI, 1.1–3.8) with HCV seropositivity or with a history of IDU (95% CI, 1.0–3.9; Table 1). KSHV was significantly elevated in HCV seropositive women with IDU

Table 1. Characteristics associated with seroprevalence of KSHV among HIV-infected pregnant women.

Characteristic	Total women (n = 887)[a]	KSHV+ [n (%)]	Odds ratio (95% CI)	P^b
Clinic site				
Chicago	208	5 (2.4)	Referent	
Boston	141	4 (2.8)	1.2 (0.3–1.5)	0.80
Puerto Rico	224	12 (5.4)	2.3 (0.8–6.6)	0.11
Houston	67	4 (6.0)	2.6 (0.7–9.9)	0.15
Brooklyn	161	9 (5.6)	2.4 (0.8–7.3)	0.11
Manhattan	86	13 (15.1)	6.1 (2.1–17.8)	0.0002
Ethnicity				
White	108	5 (4.6)	Referent	
Black	403	19 (4.7)	1.0 (0.4–2.8)	0.97
Hispanic	331	19 (5.7)	1.3 (0.5–3.4)	0.66
Other	41	4 (9.8)	2.2 (0.6–8.7)	0.24
Education less than high school				
No	438	15 (3.4)	Referent	
Yes	439	31 (7.1)	2.1 (1.1–4.0)	0.02
Annual income				
> $10,000	237	7 (3.0)	Referent	
⩽ $10 000	650	40 (6.2)	2.2 (1.0-4.9)	0.06
Age at time of delivery (years)[c]				
< 25	303	12 (4.0)	Referent	
25–28	238	13 (5.5)	1.4 (0.6–3.1)	0.41
29–33	195	12 (6.2)	1.6 (0.7–3.6)	0.26
> 33	151	10 (6.6)	1.7 (0.7–4.1)	0.21
Age at first sexual intercourse (years)				
⩾ 15	589	32 (5.4)	Referent	
< 15	291	15 (5.2)	1.0 (0.5–1.8)	0.86
Maternal HIV-1 disease class C before or during pregnancy				
No	688	39 (5.7)	Referent	
Yes	199	8 (4.0)	0.7 (0.3–1.5)	0.36
HIV-1 transmission to infant				
No	787	43 (5.5)	Referent	
Yes	99	4 (4.0)	0.7 (0.3–2.1)	0.55
Mean plasma viral load during pregnancy (copies/ml)				
⩽ 1000	131	9 (6.9)	Referent	
> 1000–3500	153	8 (5.2)	0.8 (0.3–2.0)	0.56
> 3500–10 000	194	8 (4.1)	0.6 (0.2–1.6)	0.28
> 10 000–35 000	192	11 (5.7)	0.8 (0.3–2.1)	0.68
> 35 000	204	11 (5.4)	0.8 (0.3–1.9)	0.58
Mean CD4% during pregnancy				
< 14	77	3 (3.9)	Referent	
14–28	382	21 (5.5)	1.4 (0.4–4.9)	0.56
⩾ 29	420	23 (5.5)	1.4 (0.4–4.9)	0.58
Use of antiretroviral therapy during pregnancy				
None	330	21 (6.4)	Referent	
Monotherapy	507	23 (4.5)	0.7 (0.4–1.3)	0.25
Combination	50	3 (6.0)	0.9 (0.3–3.3)	0.92
Lifetime male partners				
1	44	2 (4.6)	Referent	
2–3	249	17 (6.8)	1.5 (0.3–6.9)	0.57
4–5	179	9 (5.0)	1.1 (0.2–5.3)	0.89
6–10	189	7 (3.7)	0.8 (0.2–4.0)	0.79
⩾ 11	186	7 (3.8)	0.8 (0.2–4.1)	0.81
Oral sex during pregnancy				
No	618	33 (5.3)	Referent	
Yes	263	14 (5.3)	1.0 (0.5–1.9)	0.99
Anal sex during pregnancy				
No	819	43 (5.2)	Referent	
Yes	63	4 (6.4)	1.2 (0.5–3.3)	0.71
Types of STD during pregnancy				
Chancroid				
No	875	45 (5.1)	Referent	
Yes	9	2 (22.2)	5.3 (1.1–26.1)	0.02
Syphilis[d]				
No	800	38 (4.8)	Referent	
Yes	87	9 (10.3)	2.3 (1.1–5.0)	0.03

(continued)

Table 1. (continued)

Characteristic	Total women (n = 887)[a]	KSHV+ [n (%)]	Odds ratio (95% CI)	P^b
Genital ulcers				
No	868	45 (5.2)	Referent	
Yes	17	2 (11.8)	2.4 (0.5–11.0)	0.23
Genital warts				
No	738	34 (4.6)	Referent	
Yes	148	13 (8.8)	2.0 (1.0–3.9)	0.04
Herpes				
No	805	42 (5.2)	Referent	
Yes	81	5 (6.2)	1.2 (0.4–3.1)	0.71
Gonorrhea				
No	833	46 (5.5)	Referent	
Yes	53	1 (1.9)	0.3 (0.0–2.4)	0.35
Chlamydia				
No	762	39 (5.1)	Referent	
Yes	124	8 (6.4)	1.3 (0.6–2.8)	0.54
Trichomonas				
No	549	30 (5.5)	Referent	
Yes	338	17 (5.0)	0.9 (0.5–1.7)	0.78
Number of sexually transmitted diseases[c]				
0	341	13 (3.8)	Referent	
1	317	15 (4.7)	1.2 (0.6–2.7)	0.57
2	151	11 (7.3)	2.0 (0.9–4.5)	0.11
⩾ 3	78	8 (10.3)	2.9 (1.2–7.2)	0.04
Hepatitis C virus (HCV) serology				
Negative	700	31 (4.4)	Referent	
Positive	187	16 (8.6)	2.0 (1.1–3.8)	0.025
Recreational drug use				
None	445	24 (5.4)	Referent	
Non IDU[e]	305	9 (3.0)	0.5 (0.2–1.2)	0.11
IDU	137	14 (10.2)	2.0 (1.0–3.9)	0.04
Injection drug use/Hepatitis C virus				
IDU− HCV−	673	28 (4.2)	Referent	
IDU− HCV+	77	5 (6.5)	1.6 (0.6–4.3)	0.37
IDU+HCV−	27	3 (11.1)	2.9 (0.8–10.1)	0.11
IDU+HCV+	110	11 (10.0)	2.6 (1.2–5.3)	0.01

[a]Totals do not always sum to 887 due to missing data. [b]Chi-square or Fisher's exact test where appropriate. [c]Cochran-Armitage test for trend: $P = 0.18$ for age; $P = 0.01$ for number of STD. [d]Syphilis serology missing for 36 women, six of whom had syphilis noted upon medical record review. Three of these six were KSHV positive. The other 81 cases of syphilis were laboratory-confirmed. [e]Two-hundred and fifty-eight women reported non-injecting hard drug use and 47 reported marijuana use only. CI, Confidence interval.

(OR, 2.6; 95% CI, 1.2–5.3) and elevated although not statistically significantly among women with HCV without IDU (OR, 1.6; 95% CI, 0.6–4.3) and those with IDU without HCV (OR, 2.9; 95% CI, 0.8–10.1).

HCV seroprevalence was 80.3% among women with a history of IDU, and these two variables were highly correlated ($P < 0.0001$). Irrespective of KSHV, history of IDU and HCV seroprevalence varied significantly by clinical site and ethnicity, and it was increased with low education, older age, young age at first sexual intercourse, more male sex partners, lack of antiretroviral therapy, HIV-1 transmission to the infant, and number of STD ($P \leqslant 0.01$, data not shown).

To control confounding, women were stratified by HCV and IDU (Table 2), KSHV was non-significantly higher among non-White women without HCV or IDU (OR, 2.0; 95% CI, 0.4–20.1). Only among women with HCV seropositivity or IDU history was KSHV significantly elevated with syphilis (OR, 2.8; 95% CI, 1.0–7.9). Women with at least two STD had a non-significant increase in KSHV, irrespective of IDU and HCV seropositivity (OR, 1.9; 95% CI, 0.6–5.8 and 0.7–5.1, respectively, Table 2). With stratification for STD, KSHV seroprevalence was increased with IDU both among women with no STD and among women who had at least two STD (OR, 3.7 and OR, 2.4, respectively, data not shown).

By multivariate logistic regression (Table 3), KSHV seroprevalence was increased with IDU and HCV positivity (OR_{adj}, 3.5; 95% CI, 1.5–7.9), study site especially Manhattan (OR_{adj}, 9.8; 95% CI, 3.7–25.6), lower education (OR_{adj}, 1.8; 95% CI, 0.9–3.4), and

Table 2. Selected risk factors for KSHV prevalence in women, by injection drug use and hepatitis C virus (HCV) seropositivity.

Characteristic	Women with injecting drug use or positive for HCV			Women with no injecting drug use and negative for HCV		
	Total women (n = 214)[a]	KSHV+ [n (%)]	OR (95% CI)	Total women (n = 673)[a]	KSHV+ [n (%)]	OR (95% CI)
Clinic site						
Chicago, Boston	129	8 (6.0)	Referent	220	1 (0.5)	Referent[a]
Puerto Rico, Houston, Brooklyn	65	6 (9.5)	1.5 (0.5–4.6)	387	19 (4.9)	11.3 (1.5–85.0)
Manhattan	20	5 (25.0)	5.0 (1.5–17.4)	66	8 (12.1)	30.2 (3.7–246.4)
Ethnicity						
White	49	4 (8.2)	Referent	59	1 (1.7)	Referent
Black	88	7 (8.0)	1.0 (0.3–3.5)	315	12 (3.8)	2.3 (0.3–18.0)
Hispanic	69	7 (10.1)	1.3 (0.4–4.6)	262	12 (4.6)	2.8 (0.4–21.8)
Other	6	1 (16.7)	2.2 (0.2–24.3)	35	3 (5.4)	5.4 (0.5–54.5)
Education less than high school						
No	76	3 (4.0)	Referent	362	12 (3.3)	Referent
Yes	132	15 (11.4)	3.1 (0.9–11.1)	307	16 (5.2)	1.6 (0.7–3.4)
Age at time of delivery						
< 25	33	3 (9.1)	Referent	270	9 (3.3)	Referent
25–28	58	4 (6.9)	0.7 (0.2–3.5)	180	9 (5.0)	1.5 (0.6–3.9)
29–33	67	5 (7.5)	0.8 (0.2–3.6)	128	7 (5.5)	1.7 (0.6–4.6)
> 33	56	7 (12.5)	1.4 (0.3–6.0)	95	3 (3.2)	1.0 (0.2–3.6)
Lifetime male partners						
1–3	31	2 (6.4)	Referent	262	17 (6.5)	Referent
4–5	34	3 (8.8)	1.4 (0.2–9.0)	145	6 (4.1)	0.6 (0.2–1.6)
6–10	45	5 (11.1)	1.8 (0.3–10.0)	144	2 (1.4)	0.2 (0.1–0.9)
⩾ 11	81	4 (4.9)	0.8 (0.1–4.3)	105	3 (2.9)	0.4 (0.1–1.5)
Syphilis diagnosis						
No	163	11 (6.8)	Referent	627	26 (4.2)	Referent
Yes	41	7 (17.1)	2.8 (1.0–7.9)	46	2 (4.4)	1.1 (0.2–4.6)
Number of sexually transmitted diseases						
None	63	5 (7.9)	Referent	278	8 (2.9)	Referent
1	73	3 (4.1)	0.5 (0.1–2.2)	244	12 (4.9)	1.8 (0.7–4.3)
⩾ 2	78	11 (14.1)	1.9 (0.7–5.1)	151	8 (5.3)	1.9 (0.6–5.8)

[a]For each variable, the numbers reflect the total number of women for whom data were available. OR, Odds ratio; CI, confidence interval.

Table 3. Logistic regression analysis for KSHV seroprevalence.

Covariate	Unadjusted		Adjusted	
	OR (95% CI)	*P*	OR (95% CI)[a]	*P*
Injection drug use, hepatitis C virus status				
IDU−, HCV−	Referent		Referent	
IDU−, HCV+	1.6 (0.6–4.3)	0.35	1.7 (0.6–4.8)	0.30
IDU+, HCV−	2.9 (0.8–10.1)	0.09	2.4 (0.5–11.5)	0.26
IDU+, HCV+	2.6 (1.2–5.3)	0.01	3.5 (1.5–7.9)	0.003
Clinic site				
Chicago, Boston	Referent		Referent	
Puerto Rico, Houston, Brooklyn	2.2 (1.1–4.8)	0.04	4.3 (1.8–10.4)	0.001
Manhattan	6.7 (2.8–16.3)	0.0001	9.8 (3.7–25.6)	0.0001
Less than high school education	2.1 (1.1–4.0)	0.018	1.8 (0.9–3.4)	0.07
Number of sexually-transmitted diseases[b]	1.4 (1.1–1.9)	0.014	1.4 (1.0–1.9)	0.04

[a] Each variable was adjusted for other variables in the model. [b]In four levels (0, 1 ,2, ⩾ 3) as in Table 1. OR, Odds ratio; CI, confidence interval; IDU, injecting drug use; HCV, Hepatitis C virus.

incrementally with STD (OR_{adj}, 1.4 per STD; 95% CI, 1.0–1.9). With correction for imperfect sensitivity (90%) and specificity (97%) of the K8.1 EIA, the association between IDU and KSHV was stronger and remained statistically significant (OR, 5.53; 95% CI, 1.5–20.6).

Discussion

Our study of HIV-1-infected pregnant women in five cities in the USA and in Puerto Rico found that KSHV seroprevalence differed substantially by geography, was associated with low education and numerous

STD, and, after adjustment for these factors, was increased 3.5-fold among women with HCV infection and a history of IDU. The latter finding implies that KSHV may be transmissible by blood inoculation.

Only three (0.3%) of our 900 infants had KSHV antibodies by age 12 months, including one (2.1%) born to a KSHV-seropositive mother. These results are compatible with those reported from Sardinia [22]. They suggest that most pediatric infections occur later in childhood through close contact not only with the mother but also with others who may be shedding KSHV [12,23].

Our results in the WITS are remarkably similar to those reported by Cannon *et al.* who evaluated the HIV Epidemiology Research Study (HERS), a prospective cohort study of 1295 women in four cities in the USA [16]. WITS and HERS differ from and complement one another. The primary objectives of WITS are to understand mother-to-infant HIV-1 transmission, the influence of pregnancy on maternal HIV-1 disease, and the complications of HIV-1 infection in the offspring. By design, WITS sought to enroll all available HIV-1-positive pregnant women, irrespective of particular risk factors. In contrast, the primary objective of HERS is to identify risk factors for and complications of HIV-1 infection in women. Women were eligible for HERS only if they had engaged in IDU or prostitution or reported having sex with at least five partners, with a male IDU, or with someone known or suspected to have HIV-1 infection. As a consequence, the HERS population had much high prevalence of IDU (60%) and HCV infection (56%) than did our WITS population (15% and 21%, respectively).

Because of these differences, WITS not only corroborates the findings in HERS but also increases the generalizability of the association of KSHV with HCV or IDU. In univariate analyses, KSHV was associated with HCV in HERS (OR, 1.7; 95% CI, 1.2–2.3) and WITS (OR, 2.0; 95% CI, 1.1–3.8). As IDU was relatively infrequent in WITS, we could not examine the highly significant trend noted in HERS, in which the risk of KSHV was increased 1.9-fold with less than daily IDU and 3.8-fold with daily IDU [16]. Still, the 2-fold risk of KSHV with any IDU that we found is concordant with HERS. A third study, the Women's Interagency HIV Study (WIHS), found a non-significant association of KSHV with IDU, perhaps because they included in the referent group women who had discontinued IDU [24].

Among homosexual men, KSHV seroprevalence and seroincidence are strongly associated with more homosexual partners and syphilis [6,7,25,26]. Associations of non-syphilis STD with KSHV among homosexual men have been inconsistent [6,7,25].

In HERS, WITS, and WIHS, KSHV was approximately 2-fold higher for women with syphilis but was surely confounded by drug use. To control the confounding, HERS investigators examined syphilis and other sexual risk factors among women who denied IDU. They found KSHV to be significantly associated with engaging in prostitution (OR, 2.2) or syphilis seropositivity (OR, 2.5) but not with sexual activities or HIV-1 or herpes simplex virus type 2 seropositivity [16]. In our study, KSHV was not associated with syphilis among HCV-seronegative women who denied IDU. In contrast, KSHV was increased with syphilis (OR, 2.8) among women who acknowledged IDU or had HCV antibodies. In both groups of women, KSHV was non-significantly elevated with having at least two STD (OR, 1.9). Among all women in our study, after adjustment for HCV, IDU, geography, and education level, KSHV seropositivity was 1.4-fold more likely with each STD (Table 3). Because KSHV seroprevalence probably reflects cumulative lifetime incidence, better measures of lifetime sexual risk than we had available will be needed to better estimate the association of KSHV with STD and to control for the confounding effect of IDU.

Geographic differences in KSHV seroprevalence among women are substantial being particularly high in Manhattan and the south Bronx. KSHV was low among drug users in Amsterdam [27] and also among female and non-African male STD attenders in London [15]. Among homosexual men, seroprevalence was increased 2.7-fold and seroincidence 3.4-fold in Manhattan compared to those in Washington DC, mirroring a 7-fold higher risk of KS [7,28]. Geographic heterogeneity of KSHV seroprevalence in Italy has been well described [10,29,30].

In our study and in HERS, KSHV seropositivity was almost 2-fold higher for women who had not completed high school [16]. In contrast, ethnicity and race were not consistently associated with KSHV, independent of IDU, HCV, geography, education, and STD [16,24].

Our study has important limitations. First, there is no consensus on optimal serological testing for KSHV. On rigorous evaluation, our EIA for antibodies against the K8.1 envelope glycoprotein of KSHV had high sensitivity and specificity [18,19]. The association of IDU and HCV with KSHV was seen with either a K8.1 or an orf65 EIA in HERS [16]. A study of drug users in Amsterdam reported a very low KSHV seroprevalence (1.4% among women) and no association with IDU or HCV, perhaps because a K8.1 EIA was not used [27]. Second, with only 47 KSHV-positive women, we had limited statistical power to identify associations. More positives would have been detected with a second assay, but this would have been at the risk of blurring

associations due to non-specificity as we reported with an orf65 EIA [18]. Third, our cross-sectional analysis could identify associations but not causation. Demonstration of KSHV seroconversion following initiation of IDU, HCV infection, or a transfusion would increase the plausibility that KSHV is transmitted by blood inoculation.

In conclusion, our findings support the hypothesis that KSHV is transmitted not only by sexual or other close personal contact but also by IDU. If KSHV transmission by blood inoculation occurs, it is with low efficiency, as shown by the markedly lower seroprevalence of KSHV compared to HCV and by the inability to detect KSHV seroconversion in 32 patients who were transfused with KSHV-seropositive blood [31,32]. Both KSHV and KS are rare in people with hemophilia, suggesting that KSHV is not transmitted by plasma or plasma derivatives [4,18,33]. KSHV is highly cell-associated, so transmission via cellular transfusion, as occurs with human T-lymphotropic virus type I [34] is possible. Although the KSHV genome was not detected in the cryopreserved blood cells of any of 33 KSHV-seropositive donors [2] infectious KSHV was detected in freshly collected, stimulated lymphocytes of one healthy donor in San Francisco [35]. Because KSHV clearly causes KS and other rare diseases [36,37], the implication for protection of the blood supply is substantial.

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References

1. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, *et al.* **Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma.** *Science* 1994, **266**: 1865–1869.
2. Todd DS, Pellett PE, Watanabe KK, Sharma UK, Ablashi D, Forghani B, *et al.* **Seroprevalence of human herpesvirus 8 (HHV-8) in US blood donors using multiple serologic and PCR assays.** *53rd Annual Meeting of the American Association of Blood Banks.* Washington DC, November 2000.
3. Sitas F, Carrara H, Beral V, Newton R, Reeves G, Bull D, *et al.* **Antibodies against human herpesvirus 8 in black South African patients with cancer.** *N Engl J Med* 1999, **340**:1863–1871.
4. Gao SJ, Kingsley L, Li M, Zheng W, Parravicini C, Ziegler J, *et al.* **KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma.** *Nature Med* 1996, **2**:925–928.
5. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. **The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission.** *Nature Med* 1996, **2**:918–924.
6. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, MacRae D, Kedes DH. **Sexual transmission and the natural history of human herpesvirus 8 infection.** *N Engl J Med* 1998, **338**:948–954.
7. O'Brien TR, Kedes D, Ganem D, Macrae DR, Rosenberg PS, Molden J, *et al.* **Evidence for concurrent epidemics of human herpesvirus 8 and human immunodeficiency virus type 1 in US homosexual men: rates, risk factors, and relationship to Kaposi's sarcoma.** *J Infect Dis* 1999, **180**(4):1010–1017.
8. Frisch M, Biggar RJ, Engels EA, Goedert JJ. **Association of cancer with AIDS-related immunosuppression in adults.** *JAMA* 2001, **285**:1736–1745.
9. Ziegler JL, Katongole-Mbidde E. **Kaposi's sarcoma in childhood: an analysis of 100 cases from Uganda and relationship to HIV infection.** *Int J Cancer* 1996, **65**:200–203.
10. Whitby D, Luppi M, Sabin C, Barozzi P, Di Biase AR, Balli F, *et al.* **Detection of antibodies to human herpesvirus 8 in Italian children: evidence for horizontal transmission.** *Br J Cancer* 2000, **82**:702–704.
11. Perna AM, Bonura F, Vitale F, Viviano E, Di Benedetto MA, Ajello F, *et al.* **Antibodies to human herpes virus type 8 (HHV8) in general population and in individuals at risk for sexually transmitted diseases in Western Sicily.** *Int J Epidemiol* 2000, **29**:175–179.
12. Pauk J, Huang ML, Brodie SJ, Wald A, Koelle DM, Schacker T, *et al.* **Mucosal shedding of human herpesvirus 8 in men.** *N Engl J Med* 2000, **343**:1369–1377.
13. Viviano E, Vitale F, Ajello F, Perna AM, Villafrate MR, Bonura F, *et al.* **Human herpesvirus type 8 DNA sequences in biological samples of HIV-positive and negative individuals in Sicily.** *AIDS* 1997, **11**:607–612.
14. Kedes DH, Ganem D, Ameli N, Bacchetti P, Greenblatt R. **The prevalence of serum antibody to human herpesvirus 8 (Kaposi sarcoma-associated herpesvirus) among HIV-seropositive and high- risk HIV-seronegative women.** *JAMA* 1997, **277**:478–481.
15. Smith NA, Sabin CA, Gopal R, Bourboulia D, Labbet W, Boshoff C, *et al.* **Serologic evidence of human herpesvirus 8 transmission by homosexual but not heterosexual sex.** *J Infect Dis* 1999, **180**:600–606.
16. Cannon MJ, Dollard SC, Smith DK, Klein RS, Schuman P, Rich JD, *et al.* **Blood-borne and sexual transmission of human herpesvirus 8 in women with or at risk for human immunodeficiency virus infection.** *N Engl J Med* 2001, **344**:637–643.
17. Garcia PM, Kalish LA, Pitt J, Minkoff H, Quinn TC, Burchett SK, *et al.* **Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. Women and Infants Transmission Study Group.** *N Engl J Med* 1999, **341**:394–402.
18. Engels EA, Whitby D, Goebel PB, Stossel A, Waters D, Pintus A, *et al.* **Identifying human herpesvirus 8 infection: performance characteristics of serologic assays.** *J Acquir Immune Defic Syndr* 2000, **23**:346–354.
19. Engels EA, Sinclair MD, Biggar RJ, Whitby D, Ebbesen P, Goedert JJ, *et al.* **Latent class analysis of human herpesvirus 8 assay performance and infection prevalence in sub-saharan Africa and Malta.** *Int J Cancer* 2000, **88**:1003–1008.

20. Thomas DL, Villano SA, Riester KA, Hershow R, Mofenson LM, Landesman SH, *et al.* **Perinatal transmission of hepatitis C virus from human immunodeficiency virus type 1-infected mothers. Women and Infants Transmission Study.** *J Infect Dis* 1998, **177**:1480–1488.
21. Magder LS, Hughes JP. **Logistic regression when the outcome is measured with uncertainty.** *Am J Epidemiol* 1997, **146**:195–203.
22. Serraino D, Locatelli M, Songini M, Cirillo R, Bottazzo GF, Andreoni M, *et al.* **Human herpes virus-8 infection among pregnant women and their children: results from the Sardinia-IDDM Study 2.** *Int J Cancer* 2001, **91**:740–741.
23. Plancoulaine S, Abel L, van Beveren M, Tregouet DA, Joubert M, Tortevoye P, *et al.* **Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population.** *Lancet* 2000, **356**:1062–1065.
24. Greenblatt RM, Jacobson LP, Levine AM, Melnick S, Anastos K, Cohen M, *et al.* **Human herpesvirus 8 infection and Kaposi's sarcoma among human immunodeficiency virus-infected and -uninfected women.** *J Infect Dis* 2001, **183**(7):1130–1134.
25. Melbye M, Cook PM, Hjalgrim H, Begtrup K, Simpson GR, Biggar RJ, *et al.* **Risk factors for Kaposi's-sarcoma-associated herpesvirus (KSHV/HHV-8) seropositivity in a cohort of homosexual men, 1981–1996.** *Int J Cancer* 1998, **77**:543–548.
26. Dukers NH, Renwick N, Prins M, Geskus RB, Schulz TF, Weverling GJ, *et al.* **Risk factors for human herpesvirus 8 seropositivity and seroconversion in a cohort of homosexual men.** *Am J Epidemiol* 2000, **151**:213–224.
27. Renwick N, Dukers NHTM, Weverline GJ, Sheldon JA, Schultz TF, Prins M, *et al.* **Risk factors for human herpesvirus 8 infection in a cohort of drug users in The Netherlands, 1985–1996.** *J Infect Dis* 2002, **185**:1808–1812.
28. Goedert JJ, Biggar RJ, Melbye M, Mann DL, Wilson S, Gail MH, *et al.* **Effect of T4 count and cofactors on the incidence of AIDS in homosexual men infected with human immunodeficiency virus.** *JAMA* 1987, **257**:331–334.
29. Whitby D, Luppi M, Barozzi P, Boshoff C, Weiss RA, Torelli G. **Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy.** *J Natl Cancer Inst* 1998, **90**:395–397.
30. Vitale F, Briffa DV, Whitby D, Maida I, Grochowska A, Levin A, *et al.* **Kaposi's sarcoma herpes virus and Kaposi's sarcoma in the elderly populations of 3 Mediterranean islands.** *Int J Cancer* 2001, **91**:588–591.
31. Engels EA, Eastman H, Ablashi DV, Wilks RJ, Braham J, Manns A. **Risk of transfusion-associated transmission of human herpesvirus 8.** *J Natl Cancer Inst* 1999, **91**:1773–1775.
32. Operskalski EA, Busch MP, Mosley JW, Kedes DH. **Blood donations and viruses.** *Lancet* 1997, **349**:1327–1328.
33. Beral V, Peterman TA, Berkelman RL, Jaffe HW. **Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection.** *Lancet* 1990, **335**:123–128.
34. Manns A, Wilks RJ, Murphy EL, Haynes G, Figueroa JP, Barnett M, *et al.* **A prospective study of transmission by transfusion of HTLV-I and risk factors associated with seroconversion.** *Int J Cancer* 1992, **51**:886–891.
35. Blackbourn DJ, Ambroziak J, Lennette E, Adams M, Ramachandran B, Levy JA. **Infectious human herpesvirus 8 in a healthy North American blood donor.** *Lancet* 1997, **349**:609–611.
36. Moore PS, Chang Y. **Kaposi's sarcoma (KS), KS-associated herpesvirus, and the criteria for causality in the age of molecular biology.** *Am J Epidemiol* 1998, **147**:217–221.
37. Luppi M, Barozzi P, Schulz TF, Setti G, Staskus K, Trovato R, *et al.* **Bone marrow failure associated with human herpesvirus 8 infection after transplantation.** *N Engl J Med* 2000, **343**: 1378–1385.

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